Supplementary Material (ESI) for *Soft Matter*

Supplementary data

"Synthesis of a supramolecular aramide-peptide hybrid"

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Experimental

Materials

Technical- and most of the p. a. quality solvents were purchased from Acros Organics. Dichloromethane and DMSO were purchased from Fisher Scientific. *N*-Methylpyrrolidone (NMP) was kindly donated by BASF and stored over molecular sieve (4 Å). 9-Flourenylmethoxycarbonyl-chloride (Fmoc-Cl), N-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid beta-t-butyl ester (Fmoc-L-Asp(t-Bu)OH), trifluoroacetic acid and Wang resin were obtained by Iris Biotech GmbH, all other chemical reagents were purchased from Acros Organics and were used without further purification. Deuterated solvents (D_2O, DMSO-d_6) were purchased from Deutero GmbH.

Techniques

Standard ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker AC (300 MHz) or on a Bruker AMX 400 (400 MHz). Infrared spectra were recorded on a Nicolet 5 DXC FT-IR spectrometer. RP-HPLC analysis was performed on a Hewlett Packard HP 1090 Liquid Chromatograph equipped with PerfectSil column (MZ Analysentechnik, Mainz, Germany, 250 x 4.0 mm; 120 ODS-2 5 μm).
The samples were eluted with an acetonitrile/water gradient that started from 10 % acetonitrile rising to 90 % over a period of 35 min and maintained constant for additional 10 min. Both solvents were buffered with 0.1 % TFA. UV-detection was performed at 254 nm. Melting points were recorded on a FP 62 Mettler Toledo in a capillary tube and are uncorrected. Field desorption mass spectra were measured on a Finnigan MAT 95 and ESI mass spectra on a Micromass Q-TOF Ultima 3.
Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) measurements were performed on a Shimadzu Axima CFR MALDI-TOF mass spectrometer equipped with a nitrogen laser delivering 3 ns laser pulses at 337 nm. Sinapinic acid was used as matrix.
UV-vis measurements were accomplished on a V-630 UV-VIS Spectrophotometer and CD-spectra recorded on a JASCO J-815 CD Spectrometer.
A Philips EM 420 transmission electron microscope using a LaB_6 cathode at an acceleration voltage of 120 kV was used to obtain TEM-images. TEM grids (carbon film on copper, 300 mesh) were obtained from Electron Microscopy Sciences, Hatfield, PA, USA.

The synthesis of the oligomers was performed on an Applied Biosystems ABI 431a automated peptide synthesizer using standard Fmoc chemistry protocols. Modules A, G and E were modified for the aramide synthesis as described in detail before.[1]

Sample preparation for the TEM experiments

The peptide amphiphile **5** was dissolved in water by the addition of 6 eq. sodium hydroxide to afford a solution concentration of 1 mg/ml. The sample for TEM analysis was prepared by the drop cast method after a solution equilibration period of one week. One drop of the solution was deposited onto the carbon-coated grid, after hydrophilization of the grids for 30 s with a plasma cleaner. The samples were dried in air over night.

Synthesis of *N*-Fmoc-4-(aminomethyl) benzoic acid (N-Fmoc-PAMBA)

Aminomethylbenzoic acid (5 g, 33 mmol) and sodium bicarbonate (10.5 g, 99 mmol) were dissolved in a mixture of dioxane/water (1:1, 420 ml) and cooled to 0 °C with vigorous stirring. 9-fluorenylmethylchloroformate (8.54 g, 33 mmol) was dissolved in dioxane (14 ml) added dropwise over 15 min. The ice bath was removed and the suspension was allowed to stir at RT. After 24 h 856 ml water was added and the mixture was extracted with ether (3 x 150 mL). The aqueous layer was acidified carefully at 0 °C and with concentrated hydrochloric acid to pH 3. The resulting white precipitate was extracted into ethyl acetate. The combined organic layers were washed with water (3 x 150 ml), dried over Na_2SO_4 and concentrated in vacu0 to yield a white solid (12.3 g, 65 %).

mp 220 °C

^{1}H-NMR: δ (300 MHz, DMSO-d_6): 7.90 (m, 5 H), 7.71 (d, J=7.4 Hz, 2 H), 7.42 (t, J=7.72 Hz, 2 H), 7.34 (t, J=6.25 Hz, 4 H), 4.41 (d, J=7 Hz, 2 H), 4.26 (m 3 H).

^{13}C-NMR and DEPT: δ (300 MHz, DMSO-d_6): 167.3, 156.5, 144.9, 143.9, 140.8, 129.4 (+), 129.3, 128.2 (+), 127.6 (+), 127.1 (+), 126.9 (+), 125.2 (+), 120.2 (+), 65.3 (-), 46.9 (+), 43.6 (-).

RP-HPLC (min): 24.1 min.

IR: 3311, 3066, 2884, 2673, 2543, 1685, 1535, 1425, 1291, 1253, 757, 736.

M (FD): *m/z* (%) = 396.1 (100); 397.1 (10.7); 769.2 (5.6); 770.2 (1.6); calc.[$C_{23}H_{19}NO_4$] = 373.1

Synthesis of (*p*-benzamide)-*block*-(L-aspartic acid) conjugate

Resin functionalization and PBA_5 were accomplished as described before.[1]

The aspartic acid block was prepared by TBTU/HOBt activation using following protocol:

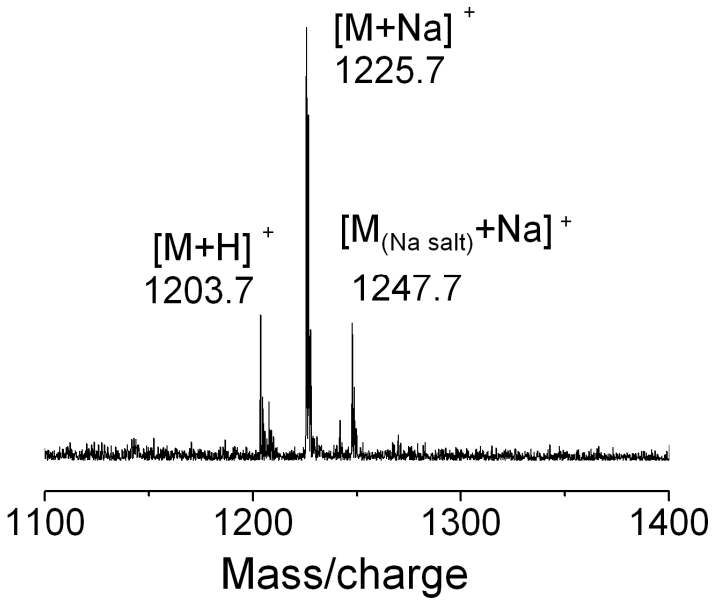
Cycle #	Repetition	Cycle description
1	4	BADEFG
5	1	BfDc

Module description for Fastmoc-protocols in automated solid phase synthesis (Applied Biosystems Peptide Synthesizer ABI 431A).

[1] Koenig, M. H.; Gorelik, T.; Kolb, U.; Kilbinger, A.F.M *J. Am. Chem. Soc.* **2007**, *129*, 704.

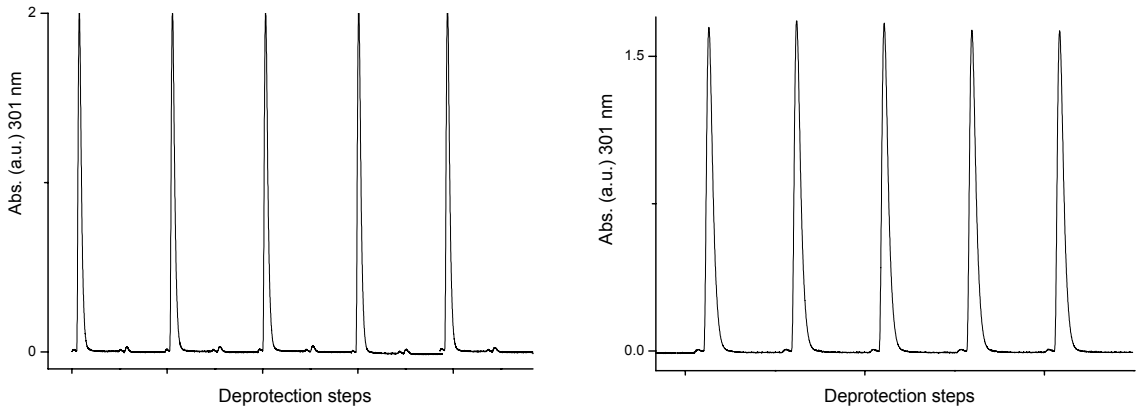
B = Fmoc-deprotection step
A = Dissolution and activation of the amino acid
D/G = Resin wash with NMP
E = Amino acid transfer
F/f = Coupling of the amino acid to the resin
c = Resin wash with DCM

The reaction time for the each Fmoc-deprotection step was 2.5 min. Cleavage from the resin was performed using 90 % TFA for 1.5 h. Azeotropic distillation of the TFA/H2O solution with toluene afford the product as a white solid (76 mg, 25%)
^{1}H-NMR: δ (300 MHz, DMSO-d_6): 10.53–10.46 (m, 4 H), 8.79 (s, 1 H), 8.29 –7.94 (m, 22 H), 7.41 (d, J = 8.5 Hz, 2 H), 4.57–4.49 (m, 3 H), 4.36 (s, 2 H), 4.01 (m, 1 H), 2.69 (m, 10 H).
^{13}C-NMR and DEPT: δ (300 MHz, DMSO-d_6): 35.99 (-), 36.07 (-), 36.16 (-), 36.47 (-), 50.05 (+), 50.13 (+), 119.42 (+), 125.36, 126.78 (+), 127.79 (+), 128.65 (+), 130.22 (+), 132.95, 142.66, 143.46, 165.25, 165.33, 165.75, 166.99, 170.47, 170.47, 65, 171.76, 171.95, 172.05, 172.08.
M(MALDI-TOF): *m/z* = 1203 $[M + H]^+$, 1225 $[M + Na]^+$, 1247 $[M_{Na\text{-}salt} + Na]^+$; calc.$[C_{56}H_{54}N_{10}O_{21}]$ = 1202



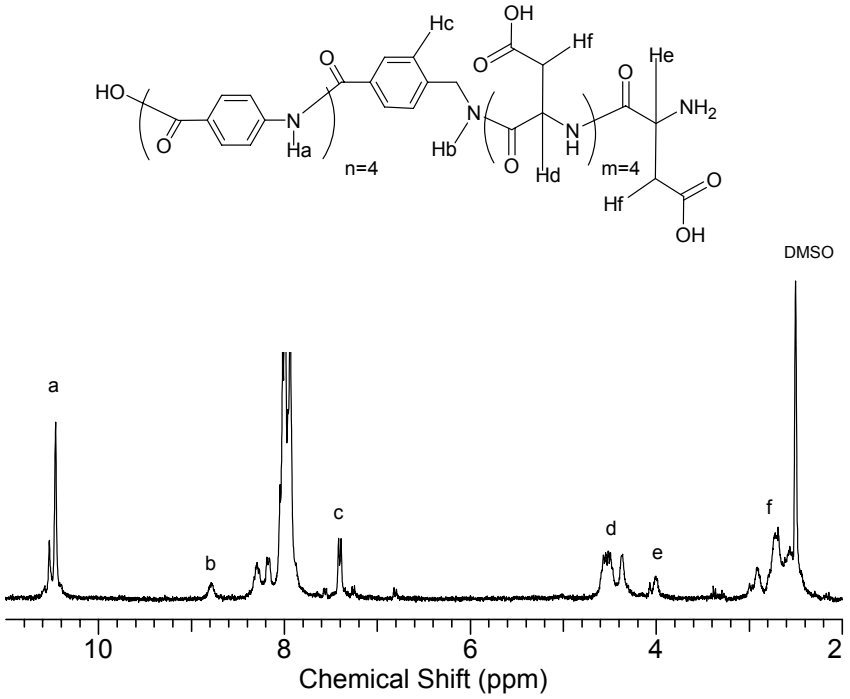
SI-Figure 1: MALDI-TOF-spectrum of peptide-benzamide conjugate 5.

Monitoring profile



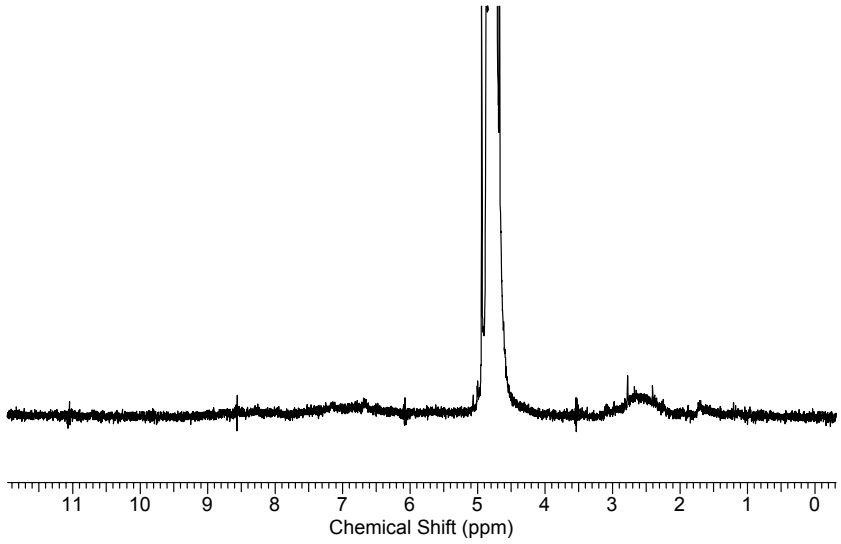
SI-Figure 2: UV-monitoring profile (λ= 301 nm) obtained for the synthesis of the aramide-(*left*) and peptide-(Asp(t-Bu)) (*right*) blocks.

Solution characterization of the aramide-peptide amphiphile

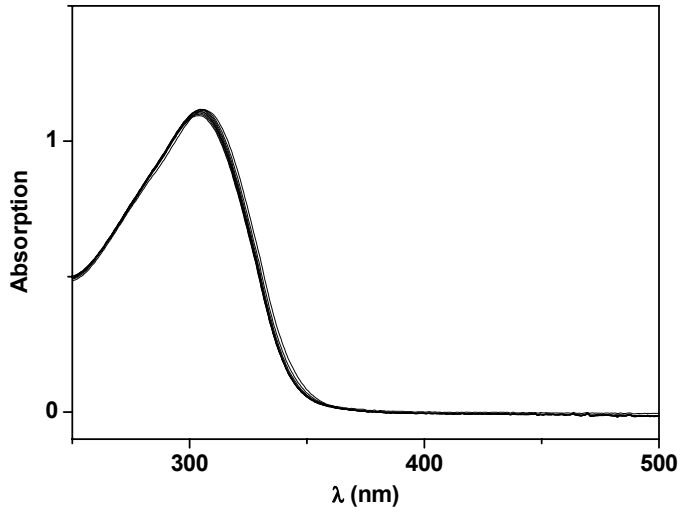


SI-Figure 3: ^{1}H-NMR spectrum of **5** in DMSO-d_6 (at least 90 % purity determined by NMR).

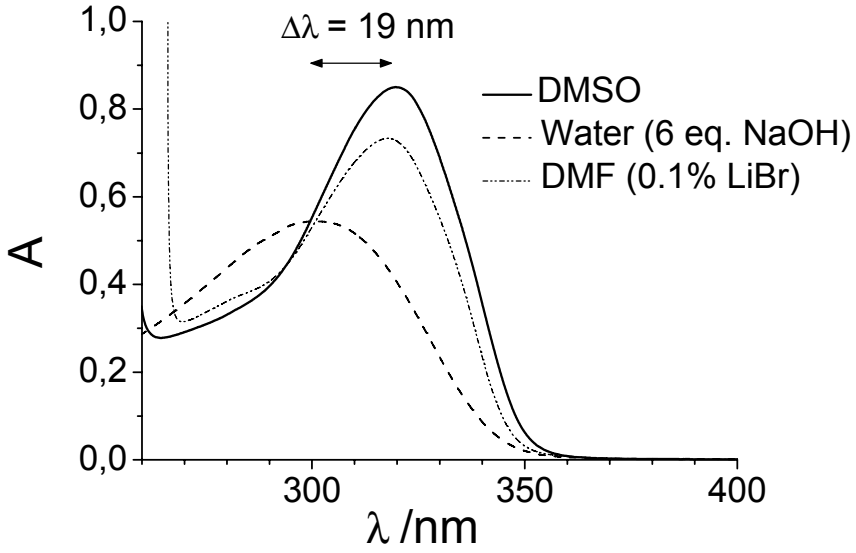
The integral of signal c is 1.8, the integral of the doublet at δ=6.8 is 0.18.
To get a lower estimate for the purity of **5** we assumed that the molecular weight of the impurity was identical to that of 5 and that the doublet at δ=6.8 corresponded to 2 aromatic protons in a para-substituted benzene. This gave a lower estimate of the purity of ca. 90%.



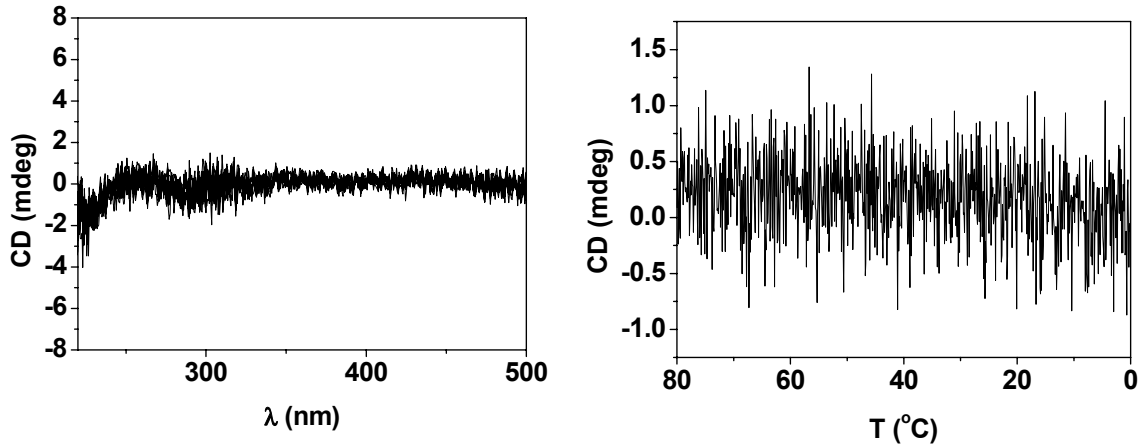
SI-Figure 4: ^{1}H-NMR spectrum of **5** in D_2O (27.7 mM in 0.17 M D_2O/$NaHCO_3$ solution).



SI-Figure 5: UV-vis absorption measurements of **5** as a function of the temperature (21 uM in 6.5 mM aq. NaOH solution). Sample cooled from 80 °C to 0 °C at a rate of 20 °C/h.

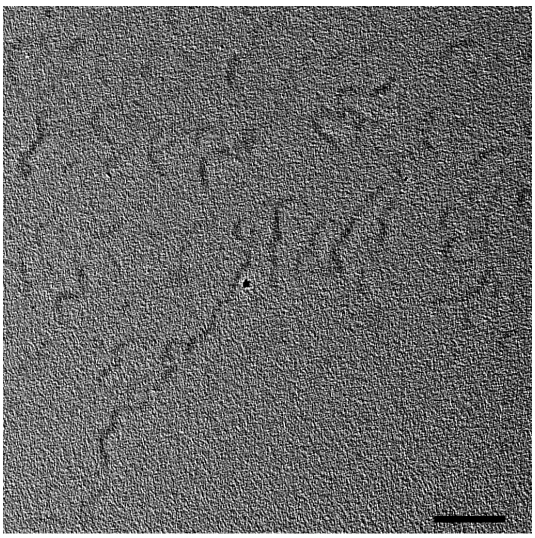


SI-Figure 6: Comparison of the UV-vis absorption spectra of compound **5** in different solvents. The spectrum in water exhibit an hypsochromic shift of the absorption maxima compared to the DMSO absorption spectrum due to aggregation. The spectrum in DMF serves as evidence, that no influences in the spectra are observed due to solvatochromic effects, as no bathochromic shift of the spectral band in DMF [2] is observed when compared to the DMSO spectrum. DMF with 0.1 % LiBr was added to enhance the solubility of **5** in this solvent.

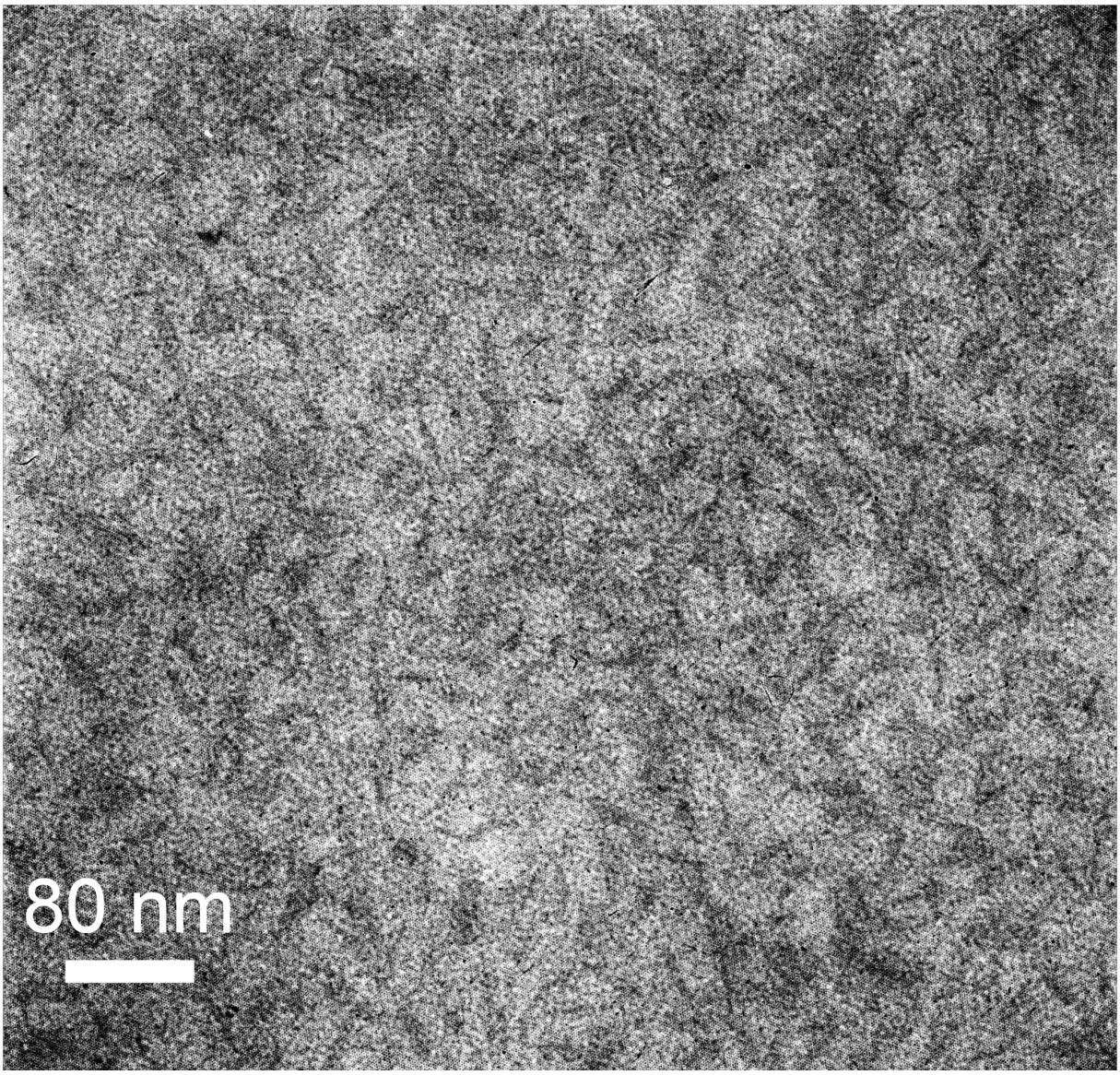


SI-Figure 7: *Left***:** CD-spectrum of **5** (21 μM in 6.5 mM aq. NaOH solution). *Right*: CD-temperature scan (300 nm).

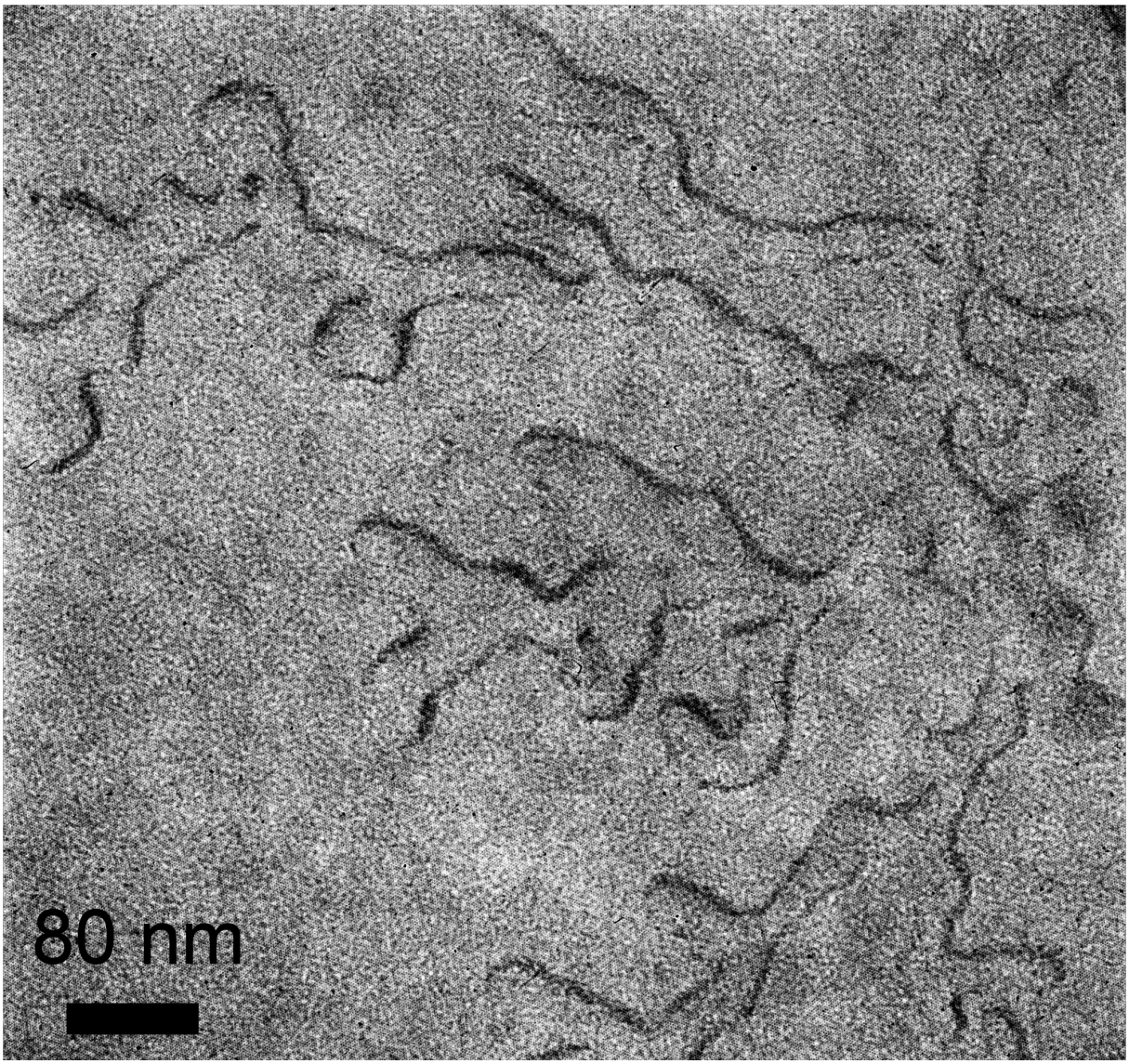
[2] The dielectric constants of the solvent were taken as a polarity parameter.



SI-Figure 8: TEM micrograph of **5** showing long worm-like nano-structures obtained from H_2O solution (6 eq. NaOH, scale bar = 100 nm). The image was not stained. Image SI-Figure 8 and Figure 2 in the manuscript were both recorded from the same TEM grid.



SI-Figure 9: TEM micrograph of **5** showing shorter tape-like nano-structures obtained from H_2O solution (6 eq. NaOH, scale bar = 100 nm). The image was stained with OsO_4.
Image SI-Figure 9 and SI-Figure 10 were both recorded from the same TEM grid.



SI-Figure 10: TEM micrograph of **5** showing longer tape-like nano-structures obtained from H_2O solution (6 eq. NaOH, scale bar = 100 nm). The image was stained with OsO_4.
Image SI-Figure 9 and SI-Figure 10 were both recorded from the same TEM grid.

Short and long tape-like structures are observed (SI-Figure 9 and 10). Yet, there should be a significant energy penalty at the ends of the micelles/tapes. This could be indicative of impurities in the system leading to a lower energy for the ends (see SI-Figure 3 for an estimate of the purity of **5**).